Oxygen transport and pO_2, pCO_2, pH of the perfusion medium and the hepatic perfusate flow during rat liver perfusion

Time (min) from the beginning of the perfusion	0	30	60	90	120
Hepatic perfusate flow (ml/g liver/min)		2.1 ± 0.4	2.1 ± 0.4	2.1 ± 0.4	2.0 ± 0.4
pO_2 (mmHg)	226 ± 45	123 ± 46	125 ± 48	132 ± 40	145 ± 40
pCO_2 (mmHg)	18 ± 6	22 ± 6	23 ± 7	23 ± 7	22 ± 7
pH	7.44 ± 0.1	7.29 ± 0.07	7.23 ± 0.09	7.19 ± 0.08	7.16 ± 0.10
Oxygen transport					
ml/g liver/h		6.3	6.3	6.3	6.0
μmol/g liver/min		4.7	4.7	4.7	4.5

Values represent means ± SD of 40 perfusions.

ber stopper. The inlet ends of the capillary tubes were connected to the needles, the outlet ends were left free. A small overpressure for the gas flow through the capillary tubes was necessary and the rubber stopper worked as an escape valve, flying off if the gas flow was hindered. The total gas flow through the two capillary tubes was 50–60 ml/min.

In other respects the perfusion system was a modification of the general principles and apparatus presented by SEGLEN and JERVELL[4]. A total perfusate volume of 50 ml was employed, consisting of 12 ml heparinized fresh rat blood, 38 ml Krebs-Ringer bicarbonate buffer solution, 50 mg D-glucose and 1.25 g bovine serum albumin (fraction V, Armour Pharmaceutical Co., Eastborne). Hemoglobin level of the perfusion medium was about 35 g/l. The perfusion pressure was 16–17 cm H_2O and the temperature 37 °C. The pump flow rate was about twice the actual hepatic perfusion flow. pO_2, pCO_2 and pH were measured by a micromethod (Combi-Analyser, L. Eschweiler, Kiel, with electrodes Eak1 for O_2, Eak2 for CO_2, and Eak3 for pH).

In the Table are presented pO_2, pCO_2, pH and oxygen transport values of the perfusion medium at the inlet side of the liver, and the hepatic perfusate flow rates of 40 perfusions, oxygenated by means of the Silastic capillary tubing. One 4-m length of the tubing was not sufficient for the oxygenation. With two 4-m lengths of the tubing the medium obtained enough oxygen to supply the oxygen consumption of the liver. Taking into account the hepatic perfusate flow rate, hemoglobin level of the medium and assuming oxygen solubility to be 0.3 ml/100 ml, hemoglobin to bind 1.34 ml O_2/g, an oxygen transport value of about 4.7 μmol/g liver/min (6.3 ml/g liver/h) by the perfusion medium, could be calculated. This exceeds the reported oxygen consumption values presented by many authors[5]. BRAUER et al.[6] have found that the oxygen consumption of the rat liver under physiological conditions was 7.8 ml/g/h and in an erythrocyte free perfusate close to 2.0 ml/g/h. Increasing the number of tubings enlarges the gas exchange surface and raises the oxygen tension in the perfusion medium, thus increasing the danger of bubble formation and changes in hepatic tissue[7]. Higher hemoglobin level in the perfusion medium increases oxygen transport, but makes the perfusion medium more expensive. CO_2 retention did not occur. The pH decreased in the course of perfusions, as it does when no special measures are taken to control pH[8].

We feel that this method is very useful in all kinds of isolated organ perfusions where a simple membrane oxygenator is needed. The system is easy to assemble, use and sterilize. Our experience is that Silastic capillary tubing is durable and re-usable for tens of perfusions. Because it does not easily kink, it may be inserted into tubes connecting different units in organ perfusions, and the need for a separate oxygenator is eliminated.

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A New Synthesis of Benzoyl Phosphate: A Substrate for Acyl Phosphatase Assay[1]

G. CAMICI, G. MANAO, G. CAPPUGI and G. RAMPONI

Institute of Biological Chemistry, University of Florence, Viale Morgagni, 50, I–50134 Firenze (Italy), 10 September 1975.

Summary. A new method for the synthesis of benzoyl phosphate was reported. The advantages are: 1. more rapid procedure; 2. lower cost; 3. higher yield.

Benzoyl phosphate has been shown to be a very useful substrate for the determination of acyl phosphatase activity. In fact it was shown that UV-absorption spectrum of benzoyl phosphate in the 220–300 nm region differs markedly from that of its hydrolysis product, i.e. benzoate and inorganic phosphate. This difference has been used by our and other laboratories[2–4] to make a continuous optical test for acyl phosphatase. Benzoyl

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Analytical data from benzoyl phosphate preparation

	Calculated for $C_6H_5 \cdot COO \cdot PO_3Li_2$ (%)	Found (%)
Hydrogen	2.35	2.64
Carbon	39.29	39.31
Lithium	6.48	6.44
Total P	14.48	15.00
Free P		0.4
Carboxyl phosphate[a]		99.6
Carboxyl phosphate[b]		101.9

[a] Hydroxylamine-ferric chloride method[5]. [b] Enzymatic method using acyl phosphatase.

phosphate has been synthesized previously as follows: 1. reaction between monosilver phosphate and benzoyl chloride; 2. isolation of silver salt of benzoyl phosphoric acid; 3. conversion into lithium salt; 4. purification of lithium benzoyl phosphate. In the present work, a simple and rapid method for benzoyl phosphate synthesis is reported.

Materials and methods. Benzoyl phosphate synthesis. This product was prepared as follows: 120 ml of water, 76 ml of pyridine, and 40 ml of 1 *M* K_2HPO_4 (40 mmol) were mixed. 20 g (90 mmol) of benzoic anhydride was added with stirring to the above solution. The reaction was carried out at room temperature until benzoic anhydride was completely dissolved (for about 10 min). Then 140 ml of 1 *M* LiOH were added and the mixture was poured into 2.4 l of cold ethanol, with stirring. After 30 min at −20°, the precipitate was collected by centrifugation, washed with cold ethanol and then with cold ether. The product was left to dry overnight under vacuum. 5.6 g of product was obtained, containing 73.5% of benzoyl phosphate.

Purification. A 2% solution of the product in cold water was adjusted to pH 6 with acetic acid. The undissolved material was discarded. Then 0.37 volumes of cold ethanol were slowly added with stirring. The suspension was kept at −20° for 30 min, then the precipitate was collected by centrifugation and washed with cold ethanol and ether. The product was about 95% pure.

The analyses described below were carried out on a product which has been further purified by the same procedure. Analytical methods. Hydrogen and carbon. The H and C content was determined by using a mod. Canal-Terzano apparatus. Carboxyl phosphate. The determination of carboxyl phosphate was carried out by 1. the hydroxylamine-ferric chloride method according to LIPMANN and TUTTLE[5]. As a reference standard benzoic anhydride was used. 2. By an enzymatic method. For this purpose we measured the extinction change of a solution of the product which was hydrolyzed completely by using a small amount of horse muscle acyl phosphatase. An extinction change coefficient at 283 nm of 0.630 mM^{-1} cm^{-1} was used[3]. Inorganic phosphate determination. The total phosphate content was determined by the method of FISKE and SUBBAROW[6]. The product was first hydrolyzed with 5 *N* H_2SO_4 at 100° for 30 min. Free phosphate was determined by the method of Baginsky et al.[7].

Lithium content. The determination was carried out by flame emission spectrophotometry using a Beckman DK-1A apparatus, at 670.8 nm. UV-spectra were obtained using a Beckman DK-1A recording spectrophotometer; IR-spectra by a Perkin-Elmer mod. 457 apparatus.

Results and discussion. In the Table the analytical data obtained for our benzoyl phosphate preparation are reported. From these data can be concluded that the preparation is > 98% pure.

As regards UV-spectra (pH 5.3), the following data have been obtained: before hydrolysis : absorption maxima at 232 and 274 nm; shoulder at 283 nm; after hydrolysis: absorption maxima at 224 and 267 nm; shoulder at 262 and 276 nm as expected[2,3]. IR-spectrum of dilithium benzoyl phosphate shows a band at 1025 cm^{-1} that has been attributed to a P-O-C(alkyl) bond[8] (the bond formed by the synthesis). It can also be noted 1. a C=O band at 1700 cm^{-1}, 2. a P=O band at 1340 cm^{-1}, 3. the absence of bands in the range 2500–2700 cm^{-1}, which confirms the absence of free —OH groups as expected for the structure of dilithium benzoyl phosphate. The product obtained after one purification step is pure enough to be used as substrate for acyl phosphatase assay.

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CONGRESSUS

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Further information by: Prof. Dr. J. A. A. Ketelaar, Chairman organizing Committee, Laboratory for Electrochemistry, van't Hoff Institute, Nieuwe Achtergracht 166, Amsterdam, The Netherlands.

CORRIGENDUM

P. PAGUIA, P. MASNER, K.-H. TRAUTMANN and A. SCHULER: *Juvenile Hormone Active Principle in Attacus atlas* L., Experientia *32*, 122 (1976). The formulae on page 122 should read correctly as follows: